Cultured Skin for Massive Burns
A Prospective, Controlled Trial

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Objective
The author compares the outcome of therapy in patients with massive burns with or without cultured autologous epithelial autografts.

Summary Background Data
The use of cultured keratinocytes has been controversial because of poor take and fragility. There have been no prospective series comparing the use of this technology with standard burn wound coverage.

Methods
During a 5-year period, 22 patients with an average burn size of 71.8% were treated with cultured keratinocytes and compared with a group of 42 controls with an average burn size of 61.6%.

Results
There was no significant difference in age, sex distribution, or third-degree component of the burn between the two groups. There was a significantly higher incidence of smoke inhalation in the cultured epithelial autograft group. There was a reduction in mortality in the cultured epithelial autograft group compared with controls, from 48% to 14% (p < 0.007). There was no difference between the two groups in other major complications, or in readmission for breakdown.

Conclusions
Coverage of patients who sustain massive burns with cultured autologous epithelial cells is an important and significant advance in management.

The Baltimore Regional Burn Center began the clinical application of cultured epithelial autografts in 1988. During that period of time, the international experience expanded from the initial reports of O'Connor to several hundred patients. There have been no controlled, prospective series. This burn center reported its initial experience with the first ten patients in 1990, and we have since continued the program. The current report contains results from 64 patients; in 22, cultured epithelial autografts were used for coverage of burns, and in 42, conventional methods of autologous skin grafting were employed. This latter group provides the controls for this series.

PATIENTS AND METHODS
All patients for entry into this study had to satisfy the following two criteria: 1) a minimum burn size of 50% with a substantial third-degree component and 2) sur-
vival beyond the first operative procedure for excision and initial coverage. This initial coverage was either excision plus early autografting, or allografting, or both. All other management modalities (resuscitation, ventilator support, topical care, etc.) were identical for all patients.

CULTURED EPITHELIAL AUTOGRAFT GROUP

In this group, a biopsy was taken from an unburned area of skin, usually within 24 hours of admission, and sent to a commercial company (Biosurface Technology (now Genzyme) Inc., Cambridge, MA) for culture. Steps were taken to minimize areas required to be covered by cultured epithelial autografts by using available donor sites as early as possible, usually within the first operative sitting. Because the cultured epithelial autografts usually were ready at the end of 3 weeks from biopsy, at the second operative sitting, excision of sites chosen to be autografted were excised and covered with fresh banked allograft, either meshed or unmeshed, which then was left in place until the third or fourth operative session, at which time cultured epithelial autograft was available. Eighteen patients in this group had cultured epithelial autografts applied to areas where the allograft had been removed completely. The last four had application of cultured epithelial autografts to areas where either the epidermis had been removed by excision or dermabrasion and the cultured epithelial autograft had been grafted on top of the engrafted allograft dermis, or, in two cases, cultured epithelial autografts were grafted onto a bed of commercially available preserved, deantigenized human allogermis (Alloderm, LifeCell Inc., The Woodlands, Texas).

CONTROL PATIENTS

The genesis of the control group is peculiar to the state of Maryland, where hospital reimbursement is regulated by state law. Although the application of cultured epithelial autografts is reimbursable by third-party payers in the state, the classification of cultured epithelial autograft as a supply prevents the hospital from accepting this reimbursement after the supply “ceiling” has been reached for each fiscal year, which often happens early in the fiscal year. Accordingly, the hospital administrator must make an annual allocation to the burn center for its budget specifically for the acquisition of cultured epithelial autografts and when this amount is expended, there are no further funds available for the remainder of that fiscal year. Because of this restriction, patients who met the entry criteria into the study but who were admitted after funding ran out constituted the control group. During the 7 years under study, in 1 year funds ran out as early as August, after which time all patients become controls; and in 2 years during the study funds were not spent completely at all. The patient population is shown in Table 1. One significant difference between the two patient populations is the burn size, which is 10% higher in the cultured epithelial autograft group than in the control population (71.8% vs. 61.6%). The third-degree component also is higher in the cultured group, but not to a significant degree. Almost all cultured patients had smoke inhalation, which we define as the need for an endotracheal intubation and ventilator support for a minimum of 3 days; 50% of controls had the same injury (p < 0.01).

STATISTICAL ANALYSIS

Groups were compared using Student’s t test, Fisher’s exact test, or chi-square analysis, as appropriate. The methodology chosen is shown in each of the results tables.

RESULTS

Clinical results are shown in Table 2. Both groups had their first operative intervention, on the average, between days 4 and 6. Cultured skin patients underwent, on the average, at least one extra grafting procedure (5.6 vs. 4.0, p < 0.018). Major complications were frequent and nearly equal in both groups; the total hospital days were significantly longer in the cultured group. The most important finding is the reduction of mortality rate from 48% in controls to 14% in the cultured epithelial autograft group, which is highly statistically significant. To our knowledge, this is the first demonstration of a reduced mortality rate in burn patients using cultured epithelial autografts. The 48% mortality rate of the control group is comparable with recently reported statistics of burn mortality; therefore, the difference in survival be-
Between cultured and control groups is not due to a falsely high mortality among controls.

Because of the often-asked question about breakdown, we further analyzed readmission of patients. There was one patient in each group who had to be readmitted for further surgery. Therefore, we believed that it was more fair to compare the average daily cost of hospitalization between the two groups. When the analysis is done this way, there is still a significantly higher difference between the two groups (possibly driven by the extra operative procedure in the cultured skin group), but the differences are no longer staggering.

The average cost of cultured skin for patients was slightly in excess of $20,000, and the average area grafted with cultured epithelial autografts was 20%; as in our original report on cultured epithelial autograft experience, the cost of coverage remains at $1,000 per percent total body surface.

**DISCUSSION**

The application of cultured epithelial autografts to massive burns continues to be extremely controversial in the literature. The “take” of cultured epithelial autografts has been reported in the range of 15% to 93.6%, with several reports between 60% and 80%, depending on exact techniques. Some authors have been extremely enthusiastic about the use of this product, particularly on very large burns; others have inconsistent experience. Histologic reports that dermal regeneration is complete at 4 to 8 years generally have not been accepted by many surgeons, who believe that the cultured epithelial autografts remain more fragile and unsatisfactory for long-term use than standard autografts; this may be substantiated by our findings of the need for increased reconstructive surgery in follow-up. There is general agreement that graft take can be improved by the use of a dermal substitute, the so-called “composite” graft. In such patients, long-term follow-up has been reported as excellent up to 4 years. We have not had much experience with this technique, and we cannot express an opinion on this subject, except to say that whether banked allograft dermis is used or a commercial dermal substitute, engraftment clearly has to be excellent before cultured epithelial autografts are applied. It is reasonable to suggest, therefore, that cultured epithelial autografts should not be applied at the same time as a dermal substitute; rather, the optimal technique seems to be to apply the allograft or dermal substitute first, protect it while it engrafts, and then prepare it for the reception of cultured epithelial autografts.

Some of the criticism of cultured epithelial autografts has centered on cost, and this is a very expensive technique. Our philosophy has been to combine standard techniques with the use of cultured epithelial autografts so that the area to be covered with cultured epithelial autografts is minimized. This not only allows the patient to benefit optimally by his own donor areas, sequentially recropped, but it also minimizes costs. On the average, our patients had two to three operative procedures before the application of cultured epithelial autografts; they

**Table 2. BALTIMORE REGIONAL BURN CENTER CULTURED KERATINOCYTE PROGRAM, 1988-1995: CLINICAL RESULTS**

<table>
<thead>
<tr>
<th></th>
<th>Cultured</th>
<th>Control</th>
<th>p Value</th>
</tr>
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<tbody>
<tr>
<td>First operating room day after burn</td>
<td>5.2 ± 0.7</td>
<td>4.7 ± 0.3</td>
<td>NS*</td>
</tr>
<tr>
<td>Total hospital days</td>
<td>96.4 ± 15.2</td>
<td>54.7 ± 2.9</td>
<td>&lt;0.014*</td>
</tr>
<tr>
<td>No. of grafting procedures</td>
<td>5.6 ± 0.6</td>
<td>4.0 ± 0.2</td>
<td>&lt;0.018*</td>
</tr>
<tr>
<td>Major complications</td>
<td>11/22 (50%)</td>
<td>25/42 (60%)</td>
<td>NS†</td>
</tr>
<tr>
<td>Mortality</td>
<td>3/22 (14%)</td>
<td>20/42 (48%)</td>
<td>&lt;0.007#</td>
</tr>
<tr>
<td>Readmission for</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STSG (breakdown)</td>
<td>1/22</td>
<td>1/42</td>
<td>NS‡</td>
</tr>
<tr>
<td>Further surgery</td>
<td>7/22</td>
<td>4/42</td>
<td>&lt;0.03‡</td>
</tr>
</tbody>
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NS = not significant; STSG = split thickness skin graft.

* Student’s t test.
† Chi square.
‡ Fisher exact.

**Table 3. BALTIMORE REGIONAL BURN CENTER CULTURED KERATINOCYTE PROGRAM, 1988-1995: FINANCIAL DATA**

<table>
<thead>
<tr>
<th></th>
<th>Cultured</th>
<th>Control</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average total cost of hospitalization ($)</td>
<td>314,874 ± 55,292</td>
<td>107,235 ± 13,802</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Average daily cost of hospitalization ($)</td>
<td>4454 ± 536</td>
<td>3311 ± 215</td>
<td>&lt;0.057*</td>
</tr>
<tr>
<td>Average cost of cultured skin/patient</td>
<td>20,280 ± 3769</td>
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* Student’s t test.
then would receive application of cultured epithelial autografts to approximately 20% of the burn, and a final operating room visit for “patching up” and closing the wound. We agree with other authors that to prepare for the application of cultured epithelial autografts, a burn wound must be perfect, uncolonized, and well vascularized. In our opinion, this degree of favorable preparation can only be carried out by preallografting the burn bed after excision, whether or not eventual allograft dermis is left in place.

SUMMARY

We report a single-center series of 22 patients who underwent cultured epithelial autografting of massive burns, with 42 controls. Despite the larger burn size and higher incidence of smoke inhalation in the cultured epithelial autograft recipients, the mortality rate was reduced significantly from 48% to 14% in this group of patients. A slightly higher reoperative rate for reconstructive surgery was necessary in the patients who had received cultured epithelial autografts. Although this technique is expensive and should not be used to the exclusion of standard techniques of burn coverage, it is an extremely useful addition to the armamentarium of the burn surgeon and provides acceptable permanent coverage.

References


Discussion

DR. ANTHONY A. MEYER (Chapel Hill, North Carolina): I would like to congratulate Dr. Munster for continuing to work on what I consider to be an interesting problem. I have several questions, some of which I think may be able to be answered fairly quickly.

The first is in terms of the definition of massive burns. You stated that anything greater than 50% would be considered. In your data, you show an average of 71% in the cultured epithelial autograft group and then 61% in the control with an incredibly small standard area of the mean or standard deviation; I could not determine any burn greater than 80% would have been included in these groups. Did you have people with that size or not elect to treat them?

Secondly, what was the operative wound bed? Were these tangentially excised? Were these excised to fascia? If so, how did that determine whether or not the patient could have regrowth out of epithelial remnants in hair follicles in a tangentially excised wound?

I realize that in your study you had a slightly greater—5.6 compared with 4—number of operative procedures when you used the cultured grafts. What was the time of coverage between the two groups? It would be hard to see how a longer time to coverage would give you a better survival, especially because it only represented 20% body surface area covered with the cultured grafts.

How do you know that the cultured skin grafts that you put on survived? Most of the reports would suggest that the survival of these grafts are at best 50%, and that late graft loss has been documented in our experience as well as in the experience reported from Brooke Army Medical Center.

In your closing comment, you made the statement that burn size does not seem to be as big an issue. But in our recent com-
parison of burn size and age, in the presence of inhalation injury, burn size remains the major predictor of survival in burn patients.

Finally, given the fact that these are not randomized but indeed represent two different groups separated by financial availability, I am not sure that we can make any conclusion that cultured keratinocytes have any proven benefit for improved survival and it would require a truly prospective study, probably in a multicenter situation.

DR. BASIL A. PRUITT, JR. (San Antonio, Texas): I compliment Dr. Munster on the good results he has achieved in the 22 patients he has treated with cultured keratinocytes. The survival results are particularly impressive in light of the fact that 20 of the cultured epithelial autograft patients had inhalation entry. Because the comorbidity of inhalation injury is strongly influenced by age and burn size, we need to know whether the distribution of those two variables, i.e., age and burn size, were comparable in the patients with inhalation injury in both groups. Because clinical signs of inhalation injury are notoriously unreliable, how did you diagnose inhalation injury and, when present, its severity?

To assess how the cultured keratinocytes exerted their beneficial effect, we need to know the time and cause of death of the patients in the control group. Because it requires 3 to 4 weeks to grow sheets of cultured cells, if the majority of deaths in that group occurred within 3 or 4 weeks of the time of the burn, one might conclude that simply taking the skin biopsy is protective. The problems that make it impossible to assess these data and evaluate the author’s conclusion are the large difference in the size of the two groups and the lack of randomization in this study. Because the possibility exists that the next 17 cultured epithelial autograft patients could all die and obliterate any mortality difference, what justification have you for having added 20 extra control subjects in whom the fatalities presumably made the observed mortality difference statistically significant?

The real question may be, why, when the LAso (extent of burn associated with a 50% mortality) for young adults is a burn of 83% of the body surface, the mortality in the control group is so high compared with the cultured epithelial autograft group? Is it possible that the lack of funds at the end of your fiscal year which allocated patients into the noncultured epithelial autograft group compromised all aspects of care?

Lastly, our experience with cultured keratinocytes has not engendered as much enthusiasm. In 19 patients to whom we applied cultured epithelial autografts on 31 occasions to an average of 11% of the body surface, the average take at 21 to 28 days after application was 31%, which represented closure of only an average of 2.8% of the body surface. The really discouraging information is evident on this slide, which indicates that the loss of cultured cell grafts was proportionate to the extent of the burn. My last question, therefore, is whether you, too, have noted this perverse inversion relationship between cultured epithelial autograft take and burn size?

DR. DAVID N. HERNDON (Galveston, Texas): The crux of this paper was astounding—a reduction in mortality in burns over half of the body from 48% to 14% in treated patients, despite an increased cost variable stated at $1,000 per percent total body surface area covered or $200,000 per patient. The medical, moral, and social implications of this work are phenomenal. This paper supports the use of cultured epidermal autografts routinely in patients with massive burns. To not do so despite increased costs, the authors contend, would triple the mortality rate.

One significant question is: Who will pay for the cultured epithelial autografts? If the hospital or burn center withholds this treatment in light of this paper and the patient dies, will the physician or hospital be held accountable?

The impact of these data requires close scrutiny. We must be satisfied in light of these contentions that the two treatment groups were truly equal. Was patient care different? Were wounds treated more aggressively in patients in preparation for the costly cultured epithelial autografts? Perhaps it is this difference in wound treatment and bias in patient care approach that accounts for the difference in mortality and not the direct use of cultured epithelial autografts.

The comparison of the two groups would be more compelling if we knew what the mortality of each group was at the time at which the cultured epithelial autograft was fully grown and ready to be applied between 3 and 6 weeks postburn. Were the mortalities in each group the same at that point? It would not be expected that cultured epithelial autografts could affect mortality that occurred before its application.

In contrast, it is general experience in other burn units that most patients who live 4 weeks postburn will go on to survive despite wound treatment, even in this massive burn category. A survival rate of 81% for burns over 50% total body surface area would not be so surprising if the analysis selects out the group of patients who have already survived 4 weeks postburn.

Is there a rigorous intent to treat this study—i.e., if a patient in the cultured epithelial autograft group died before the cultured epithelial autograft became available, would that patient be counted as a cultured epithelial autograft patient or as a control patient?

To better interpret the length of stay and cost data, more detail is required. Survivors and patients who died were combined in both groups. It is necessary to provide a comparison of survivors only and cases who died only between the two groups to make sense of these data. The comparisons given in the manuscript were between the total groups, the disproportionate numbers of deaths in the control group relative to the treatment group would affect length of stay and cost analysis.

The data presented are startling, and clearly a randomized and controlled clinical trial is mandated.

DR. DAVID M. HEIMBACH (Seattle, Washington): I share most of the same questions and critiques to which Dr. Pruitt and Dr. Herndon have alluded. I have two questions that are slightly different.

One concerns the management of the control patients. Was the whole burn excised? And if it was, was it allografted after you ran out of skin? If it was allografted, you say your allograft took very well in the cultured cell group; did it also take well in the control group? If it did, why didn’t you just leave it in place? That closes the wound. And once the wound is closed, it is closed whether it is closed with allograft or it is closed with cultured cells or it is closed with autograft. You could have then
just removed the allograft at the time when autograft was available.

The other question is an arithmetic question. You tell us that it costs approximately $20,000 per patient to cover 20% of their skin. An adult is about 1.8 m², which is 18,000 cm². Twenty percent of that, which is 20% of the body as you talk about, is 3600 cm². The cost of the cells, at least to us and to everyone else, is approximately $400 per 25-cm² container. Twenty percent, therefore, requires 144 of those little dishes, or almost $60,000. So either you are getting an awfully good deal on your skin, or you are not buying as much as might be suggested in the manuscript.

DR. PALMER Q. BESSEY (Rochester, New York): I, too, rise to congratulate Dr. Munster on his study and thank him for sharing his experience. This may help us to understand how we might use cultured skin wisely.

I also have concerns about possible selection bias. In addition to those raised by others there may be economic factors. If you have a limited budget, you are going to tend to use expensive resources on those patients who have responded well to therapy so far, be it 5, 7, or 10 days, or 3 weeks.

In addition, were there any patients who died while on the waiting list, while the skin was growing? I am also interested in the patients who needed reconstructive procedures. Was that because the scar is really quite different with the cultured skin? Or was it because the patient’s physical therapy was limited early on due to the fragility of the cultured skin?

DR. ANDREW M. MUNSTER (Closing Discussion): I am going to try to combine the answers to some of these questions, as they are overlapping.

I do have the data on the 3-week mortality of the two groups. Of the three deaths in the cultured skin group, one died before 3 weeks and two died after 3 weeks. In the control group, out of the 20 deaths, 11 died before the 3 weeks and 9 after the 3 weeks, and that is not statistically significant or different. The cause of death is the usual cause of death—a mixture of sepsis, myocardial infarction, and multiple organ failure in both groups. I could not differentiate between them.

Dr. Meyer, 50% was just the agreement I made with the hospital, that we would not graft anybody smaller than that. But in fact, in both groups, there was a range between approximately 50% to 55% all the way up to 90%. The cultured skin burn sizes seemed to be clustered together.

I have to emphasize that there is no difference in management. I operate on all of these patients. They all get excised. We excise 15% to 20% at a time and allograft immediately. And our take rate at the beginning of the cultured skin is approximately 75% because of two or three bouts of allografting after which the allograft is removed every 5 to 7 days—just like Dr. Pruitt taught me back in the old days.

We did not measure the final take. I do not think it is fair to do that unless you do the same thing on the split-thickness autograft, and that is difficult to do. Otherwise, it subjects the cultured skin to an unfair disadvantage. So really we do not know how much of it actually took, but all of these patients were excised, so these were third-degree burns.

I agree with all the discussants that the only way to solve this is a multicenter trial, but the logistics of that are mind-boggling. If somebody can design one, I would be happy to participate.

Dr. Pruitt, the diagnosis of inhalation injury, because we are an unsophisticated unit, is made by the necessity to intubate within 24 hours of admission. So all these patients were on a ventilator for a minimum of 3 days, and that unified that particular group.

About the extra controls: They really were not extra controls. The selection of admission to the study, as I mentioned before, was a burn of at least 50% and survival for the first excisional procedure, and everybody who did that was admitted to the study. And they were not controls because we ran out of money, that is how they were selected.

Intent to treat was the same for both of them. The analysis of dollar expenditures of death and the survivors, I do not have.

As far as the scarring is concerned, Dr. Bessey, I think that you are right. I have a feeling, although I do not have data on it, that in fact the cultured skin patients do scar more and require more reconstructive procedures. And I think the composite dermal epidermal skin will probably help that problem.

And finally, to answer Dr. Heimbach’s question about the arithmetic, he took me by surprise. I am going to have to go home and get my little calculator. I cannot answer that question off the top of my head.